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Adriana E. Kajon
Xiaoyan Lu *Kirsten St George*
Dean D. Erdman *Marion P. Koopmans*
Janice Louie *Taslim Allibhai*
David Schnurr *David Metzgar*

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San Diego, California 92106

MAJOR ARTICLE

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Adriana E. Kajon,[1] Xiaoyan Lu,[2] Dean D. Erdman,[2] Janice Louie,[3] David Schnurr,[3] Kirsten St George,[5] Marion P. Koopmans,[7] Taslim Allibhai,[6] and David Metzgar[4]

[1]Lovelace Respiratory Research Institute, Albuquerque, New Mexico; [2]Centers for Disease Control and Prevention, Atlanta, Georgia; [3]California Department of Health, Public Health Viral and Rickettsial Disease Laboratory, Richmond, [4]Department of Respiratory Disease Research, Naval Health Research Center, San Diego, California; [5]Wadsworth Center, New York State Department of Health, Albany; [6]Wilford Hall Medical Center (United States Air Force Lackland Air Force Base), San Antonio, Texas; [7]National Institute for Public Health and the Environment, Bilthoven, the Netherlands

Background. First isolated in the Netherlands in 1955 during an outbreak of acute respiratory disease (ARD) among military recruits, human adenovirus 14 (HAdV-14) has historically been considered rare. With no precedent of circulation in North America, HAdV-14 has been isolated from military and civilian cases of ARD of variable severity since 2003 in the United States.

Methods. Ninety-nine isolates from military and civilian cases from different geographic locations and circulation periods were characterized by restriction enzyme analysis of viral DNA and select gene sequencing.

Results. All examined viruses were found to be identical and to belong to a new genome type designated "HAdV-14p1" (formerly known as "14a"). Comparative alignments of E1A, hexon, and fiber gene sequences with other subspecies B2 HAdVs suggest that HAdV-14p1, like the closely related HAdV-11a, arose from recombination among similar HAdV-11 and HAdV-14 ancestral strains. A deletion of 2 amino acids in the knob region of the fiber protein is the only identified unique characteristic of HAdV-14p1.

Conclusion. The current geographic distribution of HAdV-14p1 involves at least 15 states in the Unites States. The role of the fiber mutations in the recent emergence of HAdV-14p1 ARD in North America warrants further study.

Human adenoviruses (HAdVs) include 52 recognized serotypes assigned to 7 species (A–G) on the basis of biophysical, biochemical, and genetic criteria [1]. Species B includes 2 genetic clusters, subspecies B1 and B2. The serotypes of subspecies B1 (HAdV-3, HAdV-7, HAdV-16, HAdV-21, and HAdV-50) generally cause acute respiratory disease (ARD), whereas the serotypes of subspecies B2 (HAdV-11, HAdV-14, HAdV-34, and HAdV-35) are more often associated with urinary tract infections and opportunistic infections of immunocompromised hosts [2].

Military recruits are highly susceptible to HAdV-associated ARD. In the absence of vaccine intervention, outbreaks are essentially continuous and result in large numbers of sick trainees, providing a unique setting to examine the molecular epidemiology and dynamics of transmission of adenovirus respiratory infection [3–5].

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Reprints or correspondence: Adriana E. Kajon, Infectious Disease Program, Lovelace Respiratory Research Institute, 2425 Ridgecrest Dr SE, Albuquerque, NM 87108 (akajon@lrri.org).

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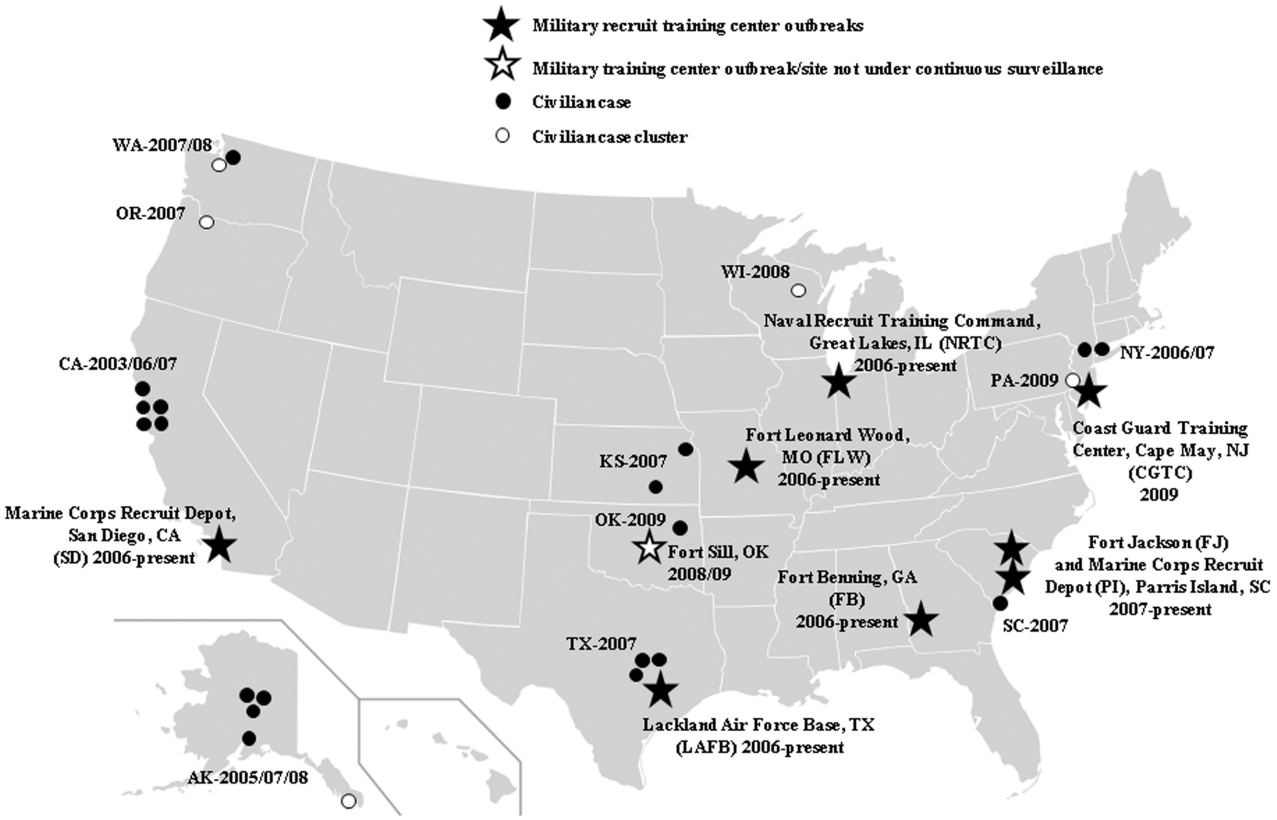

Figure 1. Geographic and temporal distribution of documented individual cases and clusters of human adenovirus 14a1 (HAdV-14a1) infections in the United States. CGTC, Coast Guard Training Center, Cape May, New Jersey; Lackland AFB, Lackland Air Force Base, San Antonio, Texas; NRTC, Naval Recruit Training Command, Great Lakes, Illinois; PI, Marine Corps Recruit Depot, Parris Island, South Carolina; SD, Marine Corps Recruit Depot, San Diego, California.

First discovered in 1955 during an outbreak of ARD at a military recruit training facility in Ossendrecht, the Netherlands, HAdV-14 (otherwise known as "agent de Wit" [6]), was subsequently isolated during similar outbreaks of disease among young adults in Great Britain in 1955 [7], Uzbekistan in 1962 [8], and Czechoslovakia in 1963 [9]. Although historically both military and civilian surveillance efforts have included assays capable of detecting and identifying HAdV-14, the circulation of this serotype was not reported between the 1960s and 2006. A few unpublished sporadic isolations in the Netherlands in the early 1970s are the only exception. Chen and colleagues [10] reported the presence of HAdV-14 in 6%–8% of all HAdVs isolated from hospitalized children in Taiwan during 2001 and 2002, but identification of these isolates was based solely on restriction analysis of polymerase chain reaction (PCR) fragments, which cannot distinguish the closely related HAdV-11 and HAdV-14.

With no precedent of previous circulation in North America, HAdV-14 was first detected between March 2006 and April 2006 among new recruits in training at 3 of the 8 military bases under continuous systematic surveillance for ARD [11]. Outbreaks of HAdV-14–associated ARD of variable severity were subsequently detected in the other 5 bases [12; the present report] and in civilian populations in Washington [13], Oregon [14], Alaska [15], Wisconsin, and Pennsylvania (as described in the present report). Isolated cases of HAdV-14–associated ARD have also been documented in several states [16, 17]. In this article, we report the results of a collaborative effort to characterize this newly emerging respiratory pathogen and inclusively document its initial spread throughout the United States.

MATERIALS AND METHODS

Surveillance of adenovirus infections among US military recruits. The Naval Health Research Center surveys US military recruit populations for ARD as part of the Global Emerging Infections Surveillance and Response System, a division of the Armed Forces Health Surveillance Center [18]. Surveillance for HAdV was established in 1996 and includes 8 training camps, as shown in Figure 1. Personnel monitor trainee pop-

ulations for symptoms of ARD (an oral temperature of ⩾38°C and either a respiratory symptom, such as cough or sore throat, or provider-diagnosed pneumonia), and clinical specimens are routinely obtained from a subset of recruits who seek medical care.

Virus strains. Ninety-nine HAdV-14 isolates were obtained from military recruits and civilian patients with ARD in multiple states. The type of clinical specimen, basic demographic data, and reported associated disease are presented for each case in Table 1. Seventy-five of the examined isolates were recovered from pharyngeal swabs collected from recruits with ARD during systematic surveillance conducted between March 2006 and March 2009. Twenty-four isolates recovered from clinical specimens obtained during convenience sampling of civilian cases of ARD were submitted to the Centers for Disease Control and Prevention, the California Public Health Viral and Rickettsial Disease Laboratory, the New York State Department of Health, or the Naval Health Research Center. Figure 1 depicts all documented clusters and discrete cases identified during the course of the study by time and date of collection. The prototype strains of HAdV-14 and HAdV-11 and a collection of respiratory subspecies B2 HAdV (HAdV-B2) strains were included in the study for comparative analysis (Table 2).

Human subjects protection. Military specimens were collected with consent under an institutional review board–approved protocol for ARD surveillance, and they were deidentified before use. Clinical samples were initially sent to the Naval Health Research Center, the Centers for Disease Control and Prevention, the California Public Health Viral and Rickettsial Disease Laboratory, or the New York State Department of Health for standard diagnostic testing. Virus isolates were provided to study personnel and coinvestigators at the Lovelace Respiratory Research Institute after deidentification. Committees at participating institutions found the protocol to be exempt of institutional review board review.

Viral DNA purification and restriction enzyme analysis. Molecular characterization of HAdV-14 isolates was conducted at the Lovelace Respiratory Research Institute and the Centers for Disease Control and Prevention. For restriction enzyme analysis, viral DNA was extracted from infected A549 cells, as described elsewhere [24]; initially digested with *Bam*HI; and further characterized by digestion with *Bcl*I, *Bgl*II, *Bst*EII, *Dra*I, *Hin*dIII, *Pst*I, *Sma*I, and *Xba*I (Promega). Fragments were analyzed by gel electrophoresis, as described elsewhere [24]. Genome types were designated in accordance with the system developed by Li and Wadell [25], which discriminates DNA variants on the basis of their *Bam*HI restriction fragment profiles and designates "p" for prototype strains and "a," "b," "c," etc, for subsequently identified variants. Additional restriction enzymes discriminate subtypes p1, p2, a1, a2, etc. In this study, novel restriction profiles were designated "N".

PCR amplification and sequencing. The E1A region, the hexon gene, or hexon hypervariable regions (HVRs) 1–7, as well as the fiber gene of the HAdV-14 and HAdV-11 strains, were PCR amplified from viral DNA or directly amplified from clinical specimens and then sequenced (primer sequences available upon request). Sequences of select HAdV-14 isolates were independently obtained at the Lovelace Respiratory Research Institute and the Centers for Disease Control and Prevention, to ensure reproducibility. The sequences were deposited in GenBank under accession numbers FJ841899–FJ841906 for hexon, FJ841907–FJ841914 for fiber, and FJ841915-FJ841922 for E1A. Previously published GenBank sequences that were confirmed in our study included HAdV-11p (NC_011202), HAdV-14p (AY803294), HAdV-34p (AY737797), and HAdV-35p (AY128640).

Sequence data were analyzed at the Lovelace Respiratory Research Institute with the use of Lasergene (DNASTAR). At the Centers for Disease Control and Prevention, Sequencher (version 4.7; Gene Codes), ClustalW implemented in BioEdit (version 7.0.5) [26], and PAUP* (version 4.0.d10) [27] were used for contig assembly, sequence alignment, and phylogenetic tree construction, respectively.

RESULTS

HAdV-14–Associated Outbreaks of ARD in US Military Recruit Training Camps

Data from continuous systematic sampling show that HAdV-14 has circulated in training facilities since 2006, affecting all 8 sites under surveillance and being detected in a significant proportion of recruits reporting with ARD. The geographic distribution and temporal occurrence of all confirmed outbreaks of ARD associated with the emerging HAdV-14 strain in basic training camps are depicted in Figure 1. The emergence of HAdV-14 at Lackland Air Force Base (San Antonio, Texas) and the Marine Corps Recruit Depot (Parris Island, South Carolina) in 2007 and at the Coast Guard Training Center (Cape May, New Jersey) in March 2009 resulted in significantly increased ARD rates and several cases of severe pneumonia, drawing specific attention from military and public health authorities. Seventy-five HAdV-14 isolates representing 8 training camps and all years of circulation were genome typed by restriction enzyme analysis. Sequences for the E1A, hexon, and fiber genes were obtained for 9 of these isolates, as indicated in Table 1. The circulation of HAdV-14 between September 2008 and March 2009 was also documented in Fort Sill, Oklahoma, but no isolates from this site were included in this study.

Table 1. North American Cases of Human Adenovirus 14 (HAdV-14)–Associated Respiratory Disease Studied

Case type, location	REA and/or sequencing performed,[a] no. of cases	Age	Sex	Specimen	Date	Premorbid condition[b]	Clinical characteristic	Outcome
Civilian								
Rural area, AK[c]	1/1	10 months	F	BAL	2005	…	Pneumonia	SW
Fairbanks, AK	0/1	7 years	F	Nasal wash	Oct 2007	…	ARD	S
Fairbanks, AK	0/1	6 months	M	Nasal wash	Nov 2007	…	Pneumonia	S
Fairbanks, AK[d]	0/2	17 years	F	Throat swab/cervical swab	May 2008	…	ARD/cervicitis	S
Prince of Wales Island, AK	0/1	48 years	M	NP/P swab	Sep 2008	…	Pneumonia	S
Fresno, CA	1/1	49 years	M	BAL	Mar 2006	COPD	Pneumonia	F
Oakland, CA	1/1	3 years	M	NP swab	Apr 2006	…	Pneumonia	SW
Oakland, CA	1/1	1.5 years	M	BAL	Apr 2006	Premature	Pneumonia	SW
Oakland, CA	1/1	4 months	M	Tracheal aspirate	Dec 2003	…	Pneumonia	S
Santa Clara, CA	1/1	55 years	M	Respiratory unknown	2007	Asthma	ARD	S
Wichita, KS	0/1	83 years	M	Bronchial wash	Jan 2008	CLL	ARD/ hypotension	F
Topeka, KS	0/1	2 months	M	Stool	May 2008	…	NA	S
New York City, NY	1/1	12 days	F	Tracheal swab	May 2006	…	Postmortem, ARD	F
New York City, NY	1/1	27 years	M	NP swab	Jan 2008	…	ARD, bone pain	S
Oklahoma City, OK	0/1	46 years	M	Lung tissue	Jan 2009	…	Pneumonia	F
Portland, OR	0/1	56 years	M	Bronchial wash	Feb 2007	…	Pneumonia	F
Parris Island, SC[e]	1/1	32 years	F	P swab	Jun 2007	…	ARD	S

San Antonio, TX	0/1	51 years	M	Conjunctival swab	Apr 2007	…	Conjunctivitis	S
San Antonio, TX	0/1	49 years	M	Respiratory unknown	Jun 2007	…	ARD	S
Rowlette, TX	0/1	21 years	M	Sputum	Jul 2007	…	Pneumonia	F
Tacoma, WA	0/1	62 years	F	Bronchial wash	May 2007	COPD	Pneumonia	S
Seattle, WA	0/1	51 years	M	NP swab	Apr 2008	Hyperten-sion, GERD	ARD	F
Gordon, WI	0/1	44 years	M	NP swab	Dec 2008	…	ARD	S
Philadelphia, PA	0/1	86 years	M	P swab	Jun 2009	…	Pneumonia	F
Military camp[f]								
MCRD, San Diego, CA	19/1	19–25 years	M	P swab	Mar 2006–Apr 2007	…	ARD	S
Fort Benning, GA	7/1	19–26 years	M	P swab	Apr 2006–Jun 2006	…	ARD	S
NRTC, Great Lakes, IL	5/1	21–24 years	M	P swab	Apr 2006–Jun 2006	…	ARD	S
Fort Leonard Wood, MO	2/1	20–36 years	M	P swab	Jun 2007	…	ARD	S
Fort Jackson, SC	2/1	22–23 years	M	P swab	May 07–Jun 2007	…	ARD	S
MCRD, Parris Island, SC	2/2	19 years	M	P swab	May 2007–Jun 2007	…	ARD	S
Lackland Air Force Base, San Antonio, TX	33/1	19–26 years	M and F	P swab	Mar 2007–Apr 2007	…	ARD/pneumonia	S and F
CGTC, Cape May, NJ	5/1	NA	NA	P swab	Mar 2009	…	ARD/pneumonia	S
Fort Sill, OK	—[f]	NA	NA	P swab	Sep 2008–Feb 2009	…	ARD	S

NOTE. ARD, acute respiratory disease (may include provider-diagnosed pneumonia at military sites); BAL, bronchoalveolar lavage; CGTC, Coast Guard Training Center; CLL, chronic lymphocytic leukemia; COPD, chronic obstructive pulmonary disease; F, fatal case; GERD, gastroesophageal reflux disease; MCRD, Marine Corps Recruit Depot; NA, not available; NP, nasopharyngeal; NRTC, Naval Recruit Training Command; P, pharyngeal; REA, restriction enzyme analysis; S, patient survived; SW, patient survived with pulmonary sequelae;

[a] Sequence obtained for E1A, hexon, and fiber genes for representative isolates.
[b] Listed, if known.
[c] Native American female infant transferred to Wilford Hall Medical Center at Lackland Air Force Base, Texas, for specialized extracorporeal oxygenation treatment.
[d] Patient first presented with a severe cervicitis and developed ARD 3 days later. Human adenovirus 14 was isolated from the cervix and respiratory tract.
[e] Military nurse on multiservice ward at Naval Hospital Beaufort, South Carolina.
[f] Multiple clinical specimens confirmed to be human adenovirus 14 at the Naval Health Research Center/not examined by REA or genomic sequencing.

Table 2. Origin and Characteristics of Subspecies B2 Human Adenovirus (HAdV) Strains Used in This Study for Comparative Analysis

B2 HAdV	Strain/year/location	Associated disease (epidemic pattern)	Author(s), year [reference]
HAdV-14p	de Wit/1957/the Netherlands	ARD (military recruit outbreak)	van der veen and Kok, 1957 [6]
HAdV-14	74-16845/1974/the Netherlands	ARD case	Not published
HAdV-11p	Slobitski/1957/USA	Poliomyelitis case	Kibrick et al, 1957 [19]
HAdV-11/14	273/1969/Spain	ARD (military recruit outbreak)	Hierholzer et al, 1974 [20]
HAdV-11a	97–26382/1997/USA	ARD (job training facility outbreak)	CDC, 1998 [21]
HAdV-11a	2474/2001/Taiwan	ARD pediatric case	Chen et al., 2004 [10]
	760/2002/Taiwan	ARD pediatric case	Chen et al., 2004 [10]
HAdV-11a	SNG1222/2005/Singapore	ARD (military recruit outbreak)	Kajon et al., 2010 [22]
HAdV-11a	QS/2006/China	ARD (civilian outbreak)	Yang et al., 2009 [23]

NOTE. ARD, acute respiratory disease; CDC, Centers for Disease Control and Prevention.

Chronology, Geographic Distribution, and Clinical Characteristics of Civilian Cases of HAdV-14–Associated Disease

Civilian cases of HAdV-14–associated ARD of variable severity have been documented in Alaska, California, Kansas, New York, Oklahoma, Oregon, Pennsylvania, South Carolina, Texas, Washington, and Wisconsin, affecting infants, young adults, and elderly individuals with and without preexisting conditions (Table 1). The inferred reconstruction of the history of circulation of HAdV-14 in the United States traced the earliest detected case of infection to California in December 2003, and the most recent cluster of cases to Philadelphia, Pennsylvania, in June 2009. The occurrence of severe pneumonia among otherwise healthy adult patients is noteworthy [13–15, 17]. Twenty-four civilian HAdV-14 isolates representing all states where cases have been confirmed were characterized by restriction enzyme analysis and targeted gene sequencing.

Genome Type Analysis of HAdV-14 and Closely Related HAdV Strains of Subspecies B2

A total of 99 HAdV-14 isolates recovered from patients with ARD between December 2003 and June 2009 were characterized by molecular methods. Genome typing was performed in comparison with the prototype strain de Wit (HAdV-14p) [6] and a collection of closely related respiratory HAdV-B2s, as described in Table 2. Because de Wit viral DNA repeatedly and reproducibly yielded difficult-to-interpret and misleading restriction fragment profiles (Figure 2), the fragment sizes predicted from genomic sequence data [28] were used as a reference for comparison. With the panel of enzymes used, restriction enzyme analysis of genomic DNA showed the Dutch HAdV-14 strain 74-16845 to be prototype-like and indistinguishable from strain de Wit. All the North American isolates of HAdV-14 were found to be identical and to belong to a new genome type distinguishable from HAdV-14p by its unique *Bst*EII and *Pst*I profiles and also by its 11a-like *Bcl*I profile (Figure 2 and Table 3). After the denomination system proposed by Li and Wadell [25], this strain was identified as corresponding to genome type 14p1. HAdV-11 strains 97–26382 (isolated in South Dakota in 1997) and SNG1222 (isolated in Singapore in 2005) yielded identical profiles with all endonucleases, corresponding to genome type 11a, which was originally described for strain BC34 isolated in China between 1965 and 1985 [29]. HAdV-11/14 strain 273 (isolated in Spain in 1969) [20] was genome typed as an HAdV-11a variant exhibiting a unique *Bst*EII profile not previously described (Figure 2 and Table 3). The relative percentage of comigrating restriction fragments (data not shown) showed HAdV-14p1 strains to be closely related to HAdV14p strains de Wit and 74-16845, but they were also very closely related to all the examined HAdV-11 strains representing variants of genome type 11a [29].

Sequence Analysis

Full-length E1A, hexon, and fiber open-reading frame (ORF) sequences obtained from geographically and temporally diverse HAdV-14p1 isolates were identical, but they differed from the reference strains included in the study in important ways.

E1A ORF. B2 E1A ORFs ranged in length from 867 to 870 nucleotides. As expected, the HAdV-14p1 E1A ORF showed higher sequence identity with HAdV-14p (99.4% nucleotides and 98.9% amino acids) than with other prototype B2 viruses, including 11p (97.2% nucleotides and 96.8% amino acids), 34p (98.6% nucleotides and 97.9% amino acids), and 35p (97.3% nucleotides and 96.1% amino acids). However, HAdV-14p1 showed even higher sequence identity with HAdV-11a strains (99.7% nucleotides and 99.6% amino acids) and HAdV-11/14 (99.6% nucleotides and 99.6% amino acids), including possession of a single 3-nucleotide GTG insertion corresponding to amino acid 148 (Ser) that was present in all HAdV-11a strains, HAdV-14/11, and HAdV-34p; an ATG (Met) insertion was found in the same position in HAdV-11p and 35p.

Hexon ORF. B2 hexon ORFs ranged in length from 2835 to 2856 nucleotides. Consistent with neutralization results, the

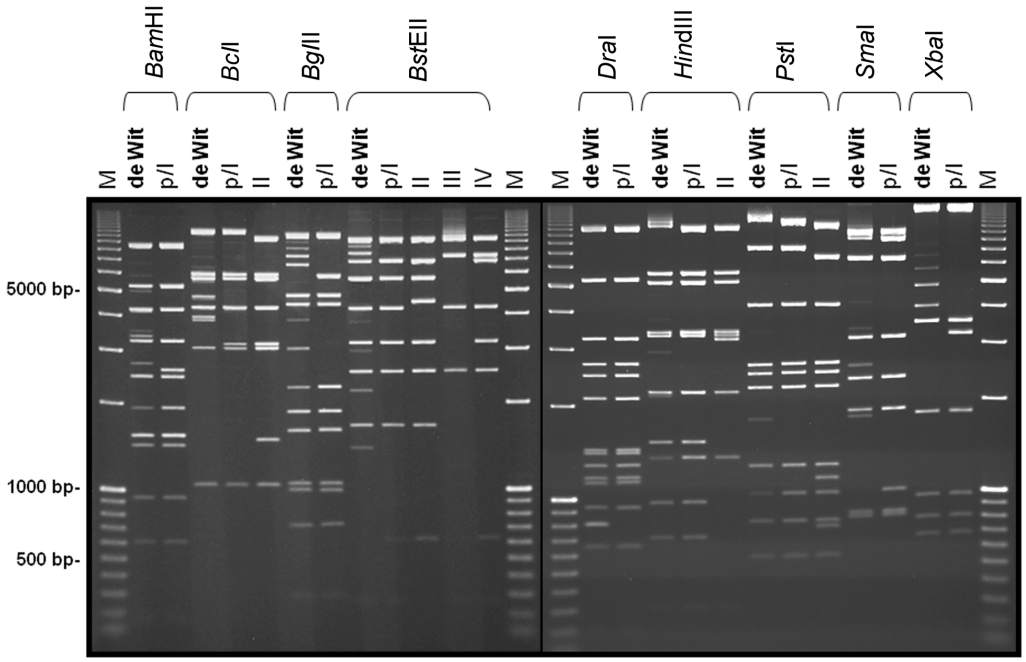

Figure 2. Restriction enzyme analysis of the emerging human adenovirus 14 (HAdV-14) strain and closely related subspecies B2 human adenoviruses. M, 1–kilobase-pair + 100-bp molecular markers. Roman numerals (I–IV) denote the different restriction profiles for each endonuclease. Aberrant profiles yielded by the prototype strain de Wit are shown for each restriction enzyme.

HAdV-14p1 hexon showed high sequence identity with HAdV-14p strains de Wit and 74-16845 (99.8% nucleotides and 99.8% amino acids), and HAdV-11a strains and HAdV-11/14 showed greater sequence identity with HAdV-11p (98.0%–98.2% nucleotides and 98.3%–98.5% amino acids) than with HAdV-14p1 (91.0%–91.3% nucleotides and 91.4% amino acids).

Fiber ORF. B2 fiber ORFs ranged in length from 969 to 975 nucleotides. The fiber of HAdV-14p1 was nearly identical to the fiber of HAdV-14p strains de Wit and 74-16845 (99.2% nucleotides), with the exception of a single 6-nucleotide deletion corresponding to amino acids 251/252 (Lys/Glu) in the fiber knob near the recognized putative receptor-binding site [30, 31] that was not detected in any of the other B2 viruses examined in this study. HAdV-11a strains, including intermediate HAdV-11/14, showed higher fiber sequence identity with HAdV-14 strains (99.2%–99.8% nucleotides and 98.7%–99.0% amino acids) than with HAdV-11p (94.0%–94.7% nucleotides and 92.2%–92.8% amino acids), consistent with the tropism of these viruses for the respiratory tract.

Phylogeny. Results of phylogenetic analysis of the E1A, hexon, and fiber ORFs of HAdV-14p1 and other HAdV-B2s are shown in Figure 3. HAdV-14p1 E1A sequences clustered more closely with those of HAdV-11a strains than with those of the HAdV-14p strains, showing high bootstrap support. All HAdV-14 hexon sequences were tightly clustered and phylogenetically distinct from HAdV-11p. HAdV-11 strains were more genetically diverse, with HAdV-11a and intermediate HAdV-11/14 forming a separate clade. The fiber sequences of HAdV-14p, HAdV-14p1, HAdV-11a, and HAdV-11/14 were highly conserved, forming a clade distinct from HAdV-11p.

DISCUSSION

Emerging infectious diseases are defined as infections that have newly appeared in the population or that have existed but are rapidly increasing in incidence or geographic range. The term "reemergence" describes the reappearance of a known disease after a decrease in incidence. Factors that can play a role in promoting or facilitating infectious disease emergence and reemergence include travel and commerce, microbial changes, and the breakdown of public health or control measures [32]. These factors are discussed in the context of the recent emergence of HAdV-14–associated ARD in North America.

Travel and commerce are likely to have played a major role in the introduction of this rare HAdV-B2 into the United States, although the routes and chains of transmission have not been identified by our efforts. The initial detection of HAdV-14p1 in cities on the US West Coast that represent major ports of entry into the country from Asia led to the intuitive hypothesis that this emerging virus was an Asian import brought to North America by international tourist and commercial travel. However, no conclusive evidence to support this hypothesis was gathered from this study or the literature. HAdV-B2 respiratory infections documented in Asia over the past 30 years have been

Table 3. Restriction Profiles of Human Adenovirus (HAdV) 14p1 and Closely Related HAdV-14 and HAdV-11 Genome Types

Species B2 HAdV, strain	Restriction enzyme								
	*Bam*HI	*Bcl*I	*Bgl*II	*Bst*EII	*Dra*I	*Hin*dIII	*Pst*I	*Sma*I	*Xba*I
HAdV-14p, de Wit/1957/the Netherlands	p/I	p/I	p/I	p/I	p/I	p/I	p/I	p/I	p/I
HAdV-14p, 74-16845/1974/the Netherlands	p/I	p/I	p/I	p/I	p/I	p/I	p/I	p/I	p/I
HAdV-14p1, Various/2003–2009/USA	p/I	II	p/I	II	p/I	p/I	II	p/I	p/I
HAdV-11/14, 273/1969/Spain	14p/I (11a[a])	II (11a[a])	14p/I (11a[a])	N[b]/III	p/I	11a/II[a]	14p/I (11a[a])	14p/I (11a[a])	14p/I (11a[a])
HAdV-11a, 97–26382/1997/USA	14p/I (11a[a])	II (11a[a])	14p/I (11a[a])	11p[c]/ IV[a]	14p/I	11a/II[a]	14p/I (11a[a])	14p/I (11a[a])	14p/I (11a[a])
HAdV-11a, SNG1222/2005/Singapore	14p/I (11a[a])	II (11a[a])	14p/I (11a[a])	11p[c]/IV[a]	14p/I	11a/II[a]	14p/I (11a[a])	14p/I (11a[a])	14p/I (11a[a])
HAdV-11p, Slobitski/1957/USA	11p[c]	11p[c]	11p[c]	11p[c]	ND[d]	11p[c]	11p[c]	11p[c]	11p[c]

NOTE. Roman numbers denote the distinct restriction profiles illustrated in Figure 2.

[a] Restriction profiles described by Li and colleagues [29] for genome type HAdV-11a, strain BC34.

[b] Novel profile not described by Li and colleagues [29].

[c] Restriction profiles described by Li and colleagues for HAdV-11p, strain Slobitski [29].

[d] Not described by Li and colleagues [29] or by the present study.

attributed to HAdV-11 [33–36]. The Taiwanese HAdV-B2 isolates examined in the present study were originally reported as HAdV-14 [10], but they proved to be HAdV-11a, with hexon, fiber, and E1A region sequences almost identical to those obtained for other Asian HAdV-11a strains [23, 37]. HAdV-14 was isolated in 1955 in the Netherlands, during an outbreak of ARD among recruits in training [6]. During the next decade, the newly described serotype was detected during similar military and civilian outbreaks in Europe [7–9], but it remained virtually absent both in routine adenovirus surveillance samples and in specimens collected worldwide in the following 40 years, indicating either its disappearance, its circulation in very restricted niches, or its association with disease so mild that it did not require medical attention or merit further investigation. The only exceptions were previously unreported sporadic isolations in the Netherlands, where the virus was last detected in 1974.

In spring 2006, the US Department of Defense detected cases of HAdV-14 infection among military recruits in several training facilities, during continuous surveillance of HAdV-associated ARD. The examination of archived records of HAdV isolation by the California Public Health Viral and Rickettsial Disease Laboratory, the Centers for Disease Control and Prevention, and others [14] traced back the circulation of this serotype in North America to California in December 2003. The most recent activity of this virus in civilian communities was documented in Pennsylvania in July 2009. The emergence of HAdV-14 at the geographically close Coast Guard Training Center in March of 2009 confirms the nationwide rapid dissemination of this virus in both military and civilian populations. All examined isolates representing a diversity of sources and periods of circulation are essentially identical, and they belong to a new genomic variant designated "14p1" (formerly known as "14a" [17]). In this study, the close similarities identified between HAdV-14p1, the Dutch HAdV-14p strains de Wit and 74-16845, and the HAdV-11a variant isolated during the 1969 ARD outbreak in Spain, together with published records of the geographic distribution of HAdV-14 since its discovery, suggest a possible European origin for the emerging virus that should be further investigated by analysis of additional isolates of confirmed serotype identity.

Microbial change and evolution have contributed to the emergence and reemergence of viral infectious disease [38]. Our data suggest that HAdV-14p1 acquired the E1A gene from an 11a-like virus through recombination but that it has otherwise remained evolutionarily stable in the hexon and fiber genes, with the exception of the short unique deletion found in the fiber knob. Our findings also suggest that HAdV-11a strains arose from recombination events between similar ancestral viruses, bringing together 14-like E1A and fiber genes with an 11-like hexon. The detailed mapping of the differences between these viral genomes is in progress. The 6-bp deletion in the knob region of the fiber gene of HAdV-14p1 is absent in all other HAdV-11 and HAdV-14 strains examined in this study, and it is so far the only distinct feature identified for the emerging virus. Wang and colleagues [39] recently showed that the fibers of HAdV-14p and HAdV-14p1 do not bind to CD46, and they demonstrated the use of the same (still unknown) receptor for both viruses. The host cell range and relative affinity of HAdV-14p1 for cellular receptors should be further examined to determine the significance of these unique mutations.

The development of pneumonia in infected young adults with no apparent preexisting conditions is intriguing and has been considered a possible indicator of a higher pathogenicity for this emerging strain of HAdV-14. However, it is likely that other important host and/or environmental factors are implicated in determining the outcome of adenovirus infection in this age group. With the exception of the Lackland Air Force Base and the Coast Guard Training Center, where more severe

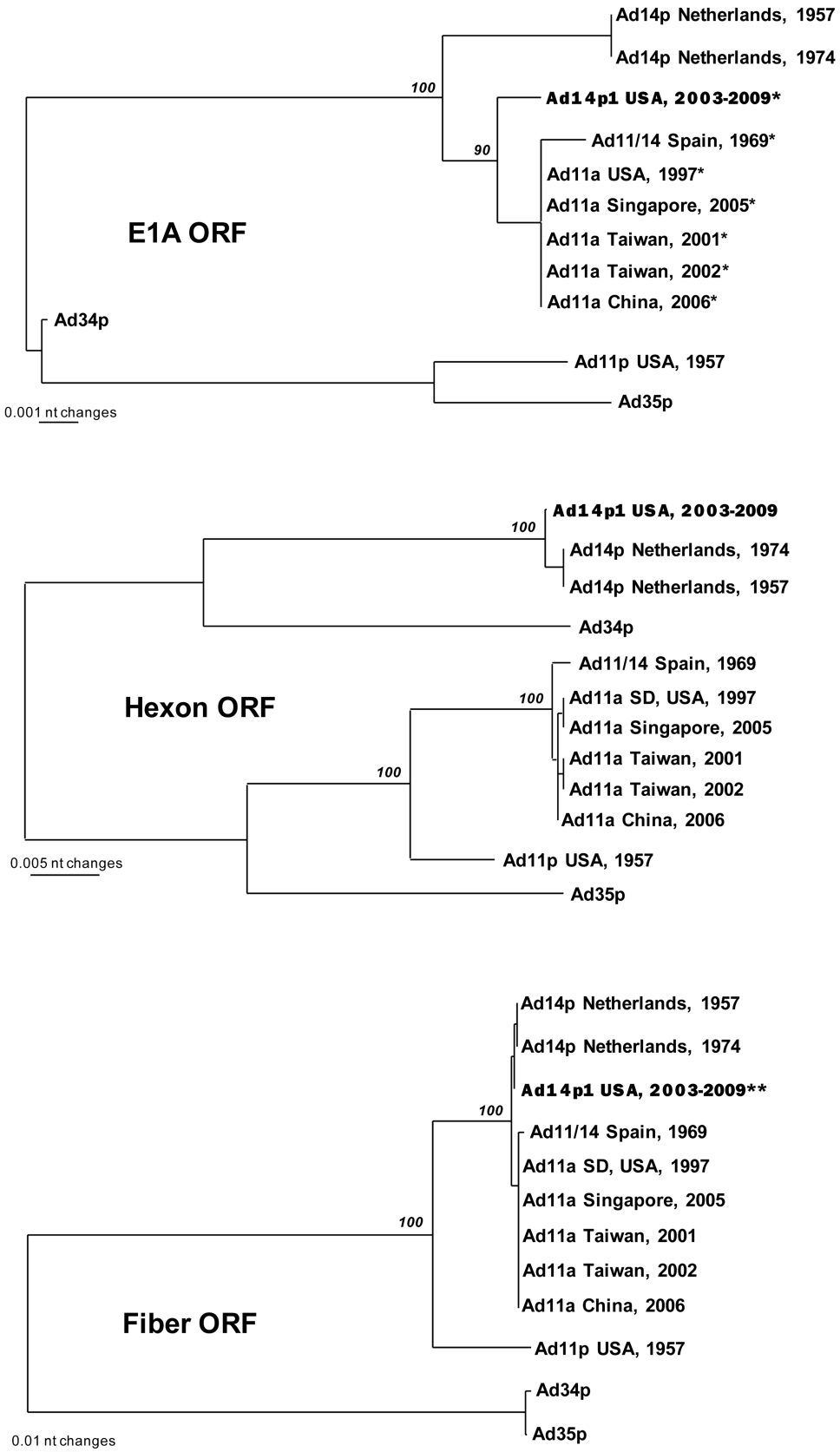

Figure 3. Midpoint-rooted neighbor-joining trees of the full-length E1A, hexon, and fiber open-reading frames (ORFs) of B2 human adenovirus (HAdV) prototype strains (11p, 14p, 34p, and 35p) and field isolates (11a, 11/14, 14p, and 14p1). Numbers at selected nodes are percentages of 1000 bootstrap replicates. Asterisks (* and **) denote unique indels common to the designated strains that were not weighted in the analysis. nt, nucleotides; SD, South Dakota.

disease has been documented (in the present study and in [12, 13]), HAdV-14 infections have not resulted in recruit morbidity higher than that caused by other serotypes. Using the available data, it is hard to establish whether HAdV-14p1 is more virulent than other species B HAdVs. The lack of permissive animal models of HAdV respiratory infection represents a mayor limitation to the scope of experiments that can be designed to study HAdV-14p1 pathogenesis and transmission.

The reemergence of HAdV infections among recruits in training over the past decade [4, 5] is undoubtedly the result of the interruption of vaccination in 1996. Formulated to target only HAdV-4 and HAdV-7, the vaccine successfully reduced the burden of HAdV-associated ARD for >25 years. The discontinuation of its use resulted in the rapid increase in the rates of HAdV-associated ARD and in the reemergence of HAdV-4 as the predominant causative agent of ARD in the military recruit environment [4, 40]. After 7 years of almost-complete dominance of this niche by HAdV-4, the simultaneous reemergence of diverse species B HAdVs, including the vaccine-targeted HAdV-7 as well as HAdV-3, HAdV-21, and HAdV-14, was detected by routine surveillance in 2006 [11]. Whereas the association of species B1 HAdV-3, HAdV-7, and HAdV-21 with civilian respiratory illness in the United States in the past decade has been consistently documented [41–44] (A.E.K., personal communication), the detection of HAdV-B2s in association with ARD in immunocompetent individuals rarely has been reported in North America. An HAdV-11–associated outbreak that occurred at a job training facility in South Dakota in 1997 and that had epidemiologic features similar to those of an outbreak at a typical boot camp [21] is probably the only well-documented episode.

The currently available data show that the distribution of confirmed HAdV-14p1–associated ARD involves 15 US states but no Canadian provinces [45], predicting further dissemination of the virus and the occurrence of new cases of respiratory illness. Seroprevalence studies in progress will determine the role of preexisting immunity as a facilitator of traffic and dissemination of HAdV-14 into new susceptible populations. Preliminary data show extremely low antibody titers against HAdV-14 in recruits sampled at admission to training camp [12] (D.M., personal communication).

The similarity between HAdV-11a–like and HAdV-14 genomes explains the limitations of some approaches to serotype without conducting neutralization assays or hexon gene sequencing [46, 47]. The ability to identify HAdV-B2 isolates at the serotype level and to determine the sequence of their fiber genes will be critical for tracking the dissemination of HAdV-14p1 from current sites of circulation and for the long-term understanding of the natural history and transmission of species B HAdV infections.

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14. ABSTRACT (maximum 200 words)

First isolated in the Netherlands in 1955 during an outbreak of acute respiratory disease (ARD) among military recruits, human adenovirus 14 (HAdV-14) has historically been considered a rare respiratory HAdV. With no precedent of circulation in North America, HAdV-14 has been isolated in the United States since 2003 from military and civilian cases of ARD of variable severity. Genomic analysis of isolates representing different locations and circulation dates showed all examined viruses to be identical and to belong to a new genome type designated HAdV-14a1. The alignment of E1A, hexon, and fiber gene sequences with those of other B2 HAdVs suggests that HAdV-14a1 has arisen from recombination events between HAdV-11 and HAdV-14p strains. A 6 base pair deletion near the predicted binding site of the fiber gene is, so far, the only distinct genetic characteristic of HAdV-14. The current documented geographic distribution of HAdV-14a1-associated ARD involves 14 US states but no Canadian provinces.

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